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Characterization and evaluation of microsatellite loci suitable for studies on mating system, parentage, and genetic identity in red titi monkeys (*Callicebus discolor*) and saki monkeys (*Pithecia aequatorialis*)

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Characterization and evaluation of microsatellite loci suitable for studies on mating system, parentage, and genetic identity in red titi monkeys (*Callicebus discolor*) and saki monkeys (*Pithecia aequatorialis*)

by

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Thesis

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Arts

The University of Texas at Austin

May 2015

Acknowledgements

I would like to express my deepest thanks to my advisor, Dr. Anthony Di Fiore, for his advice and support in all steps of this study and my graduate career. I am grateful to have had the opportunity to work with Dr. Di Fiore's titi and saki monkey samples in his laboratory. I would also like to thank Dr. Deborah Bolnick for her valuable comments on my thesis. I am extremely grateful to Dr. Bolnick for her patience and constant support when I had methodological issues and questions about genetics. I am truly thankful to my colleagues in the Department of Anthropology for their support during my first years in graduate school. I am especially grateful to Laura Abondano for her valuable encouragement and support with my study's methods. Finally, I would like to express my deepest gratitude to my family in Brazil, my husband, and my sweet daughter for their love, constant encouragement, patience, and respect.

Abstract

Characterization and evaluation of microsatellite loci suitable for studies on mating system, parentage, and genetic identity in red titi monkeys (*Callicebus discolor*) and saki monkeys (*Pithecia aequatorialis*)

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The University of Texas at Austin, 2015

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Among the pitheciids (Primates: Platyrrhini), titi (*Callicebus*) and saki (*Pithecia*) monkeys are both described as having a monogamous mating system. Titi monkeys live in small groups comprising a male-female pair and their offspring. Males provide a high level of parental care, and pair-mates show a high degree of synchronization and social contact. The composition of saki groups varies, from pair-mate units to small multimale-multifemale groups. Males and females present a much lower degree of social coordination; and among saki males, parental care is relatively absent. The term 'monogamy' is currently used to refer to both the male-female pair living arrangement (social monogamy) and the pattern of both males and females mating and breeding with a single member of the opposite sex (genetic monogamy). However, whether social monogamy typically (or ever) reflects genetic monogamy is an important question that arises when studying socially monogamous primates. To evaluate this question, molecular markers capable of being used to assess genetic parentage are critical.

Identifying and characterizing polymorphic genetic markers for titi and saki monkeys is thus important for studies of their mating system. In this study, I evaluated the utility of 13 microsatellite marker loci for parentage determination in *Callicebus discolor* (red titi monkey) and *Pithecia aequatorialis* (equatorial saki monkey) from the Tiputini Biodiversity Station in Ecuador. Microsatellite parameters and parentage analyses were conducted using the softwares Arlequin v3.11 and Cervus v3.0.3. I successfully identified a panel comprising 10 and 12 polymorphic microsatellite loci for titis and sakis, respectively, by screening these markers in a set of 17 titi and 10 saki individuals from 5 social groups that have been the subjects of behavioral observation in the field for up to 11 years. These panels amplified reliably and provided a combined parentage exclusionary percentage of 96.7% and 98.5% when both parents were unknown, and of 99.7% and 99.9% when one parent was known for titis and sakis, respectively. With these marker panels, I successfully assigned maternity for 4 titi monkeys and 3 saki offspring, and paternity to 8 titis and one saki offspring. All but one of the parentage assignments were consistent with exclusion-based parentage. Additionally, all but two parentage assignments were in accordance with expectations based on observational fieldwork. The genetic parentage results, albeit preliminary, suggest that both panels of loci selected and characterized here will be useful for future studies on mating systems, kin selection, relatedness, and other population genetic studies of both taxa.

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Introduction

Based on both morphological and molecular evidence, the New World Monkeys (Infraorder Platyrrhini) can be divided into three families: the Atelidae (howlers, spider monkeys, woolly monkeys, and muriquis), the Cebidae (capuchins, squirrel monkeys, tamarins, marmosets, and owl monkeys), and the Pitheciidae (sakis, uacaris, and titi monkeys) (Barroso et al. 1997, Opazo et al. 2006, Osterholz et al. 2009, Perelman et al. 2011, Ray et al. 2005, Schneider et al. 2001, Wildman et al. 2009). The Family Pitheciidae is considered to be the most basal lineage within the platyrrhines (Herke et al. 2007, Hodgson et al. 2009, Perelman et al. 2011, Wildman et al. 2009), and its monophyly is supported by seven *Alu* insertions (Osterholz et al. 2009).

The Family Pitheciidae includes the genera *Pithecia* (sakis), *Chiropotes* (bearded sakis or cuxiús), *Cajajao* (uakaris), and *Callicebus* (titi monkeys) (Barroso et al. 1997, Harada et al. 1995, Rosenberger et al. 1990). Like many other New World Monkeys, pitheciids are primarily frugivorous, but several genera show a preference for unripe fruits (Kinzey & Norconk 1993, Mittermeier & van Roosmalen 1981, Van Roosmalen et al. 1988). Unlike most other platyrrhines, they present varying degrees of specialization for sclerocarpic harvesting and seed predation (Ayres 1989, Barnett 2013, Kinzey & Norconk 1990, Ledogar et al. 2013, Peres 1993, Van Roosmalen et al. 1988). These dietary specializations are reflected in their dental morphology, which distinguishes most of the living pitheciids from other extant platyrrhines. Members of the Subfamily Pitheciinae, *Chiropotes*, *Cacajao* and *Pithecia*, have canines and molars that are adapted to this specialized diet of unripe seeds and fruits with very hard pericarps (Kinzey 1992, Ledogar et al. 2013), while the basal taxon *Callicebus* (Subfamily *Callicebinae*) shares

some of the seed-eating habits of pitheciines, but to a much lesser degree (Heiduck 1997, Kinzey 1977, Müller 1996, Palacios et al. 1997, Palacios & Rodríguez 2013) and without the extreme dental adaptations observed in sakis, bearded sakis, and uakaris (Ledogar et al. 2013).

Pitheciids are small to medium sized primates (850 to 3,500g in body weight) (Boubli et al. 2008, Ford 1994, Ford & Davis 1992, Norconk 2011, van Roosmalen et al. 2002). Sexual dimorphism in body mass and canine weight is minimal in titis and ranges from low to moderate in sakis, bearded sakis, and uakaris, wherein males are slightly larger than females (Plavcan 1999). Group sizes vary from small social groups with small home ranges observed in titis and sakis (e.g., from 2 to 7 individuals in the pair-bonded *Callicebus* and from 2 to 12 individuals in *Pithecia*) to relatively large group sizes observed in bearded sakis and uakaris (Norconk 2011).

GENUS *CALLICEBUS* THOMAS, 1903 AND *CALLICEBUS DISCOLOR* (I. GEOFFROY & DEVILLE, 1848)

The titi monkeys are a diverse group of Neotropical primates, which present a wide geographic distribution in South America. *Callicebus* is one of the most speciose of all primate genera, and its taxonomy has been reviewed by several authors (Groves 2001, Hershkovitz 1988, Hershkovitz, P. 1990, Kobayashi 1995, Kobayashi & Langguth 1999, van Roosmalen et al. 2002). The genus was previously divided into four species groups (Hershkovitz 1988), but the most recent taxonomic revision recognizes 5 groups – the *donacophilus*, *cupreus*, *moloch*, *torquatus*, and *personatus* groups. In terms of species-level diversity, Hershkovitz (1988) divided the genus into 13 species and 24 taxa, thus considering most of the recognized forms of the titis as subspecies. Most recently, van

Roosmalen et al. (2002) elevated all recognized forms (28) to the species level. Since the last broad taxonomic review of the genus by van Roosmalen et al. (2002), another four putative new species have been described (Dalponte et al. 2014, Defler et al. 2010, Gualda-Barros et al. 2012, Wallace et al. 2006). The subject of this study – the red titi monkey – is currently referred to by the species name *Callicebus discolor* and is classified within the *C. cupreus* group (Kobayashi & Langguth 1999, van Roosmalen et al. 2002).

Titi monkeys are found mainly in tropical forests of the Amazon and Orinoco river basins, but their ample geographic distribution also includes the Atlantic forest region of Brazil and the Chaco and dry forests of Paraguay and Bolivia as habitats (van Roosmalen et al. 2002). Titis are quite variable in their habitat requirements (Bicca-Marques & Heymann 2013). They are found in a variety of forest types, including both primary and secondary forests (Chagas & Ferrari 2010, Trevelin et al. 2007, Wagner et al. 2009) and both *terra firme* and flooded forests (Defler 1994, van Roosmalen et al. 2002), and the annual rainfall in their habitats varies from 500 mm in the Brazilian Caatinga (e.g., *C. barbarabrownae*: Marinho-Filho & Veríssimo 1997) to more than 3000 mm in the Ecuadorian Amazon (e.g., *C. discolor*: Di Fiore et al. 2009). Additionally, some species show resistance to certain degrees of habitat disturbance (e.g., C. *donacophilus*: Pyritz et al. 2010).

Titi monkeys are small body size primates with an overall size of 270-450mm from head to rump (Hershkovitz, P. 1990) and weight of 0.8 to 1.38 kg (Smith & Jungers, 1997). They live in small family groups typically consisting of an adult male, an adult female, and 1 to 3 offspring (infants and juveniles) (Bicca-Marques & Heymann 2013, Wright 1986). *Callicebus*, as well as *Aotus* (the owl monkeys), are typically described as socially monogamous primates based on behavioral and genetic data (Huck et al. 2014,

Kinzey & Wright 1982, Wright 1986). Strong bonds between the pairmates of titi monkeys have been reported based on their affiliative behaviors (e.g., grooming, huddling, tail twining), their emotional reaction to separation from the pair-mate, and the observation that specific vocalizations occur most frequently between the male and female of a pair (Anzenberger 1988, Fernandez-Duque et al. 1997, 2000; Kinzey & Wright 1982, Moynihan 2009, Robinson 1981). Additionally, among titi monkeys, mature males play an active role in parenting and manifest caring behaviors towards the infants like carrying, grooming, playing, and huddling (Fernandez-Duque et al. 2009, 2013; Fragaszy et al. 1982).

The red titi monkey occurs in Peru, Colombia, and Equador (Hershkovitz, P. 1990). Two pelage characteristics differentiate red titi monkeys from other species in the genus: a blaze of white fur across the forehead, which contrasts with an upper dark-brown band also in the forehead, and the reddish chest and belly, which contrasts with the agouti back and sides of the body (Hershkovitz, P. 1990, van Roosmalen et al. 2002). Like other titi monkeys, *C. discolor* appears to live in socially monogamous groups and males provide much of the offspring care (Van Belle et al. in review; Porter et al. in review).

GENUS *PITHECIA* DESMAREST, 1804 AND *PITHECIA AEQUATORIALIS* (HERSHKOVITZ, 1987)

Saki monkeys, genus *Pithecia*, are also widely distributed in the tropical forests of South America, from the Guiana Shield in the north to the foothills of the Andes in Ecuador and Peru to the west, to northern Bolivia in the south, and throughout the

Amazon Basin in Brazil (Hershkovitz 1987, Marsh 2014). Hershkovitz (1987) arranged the sakis in two groups, the *Pithecia pithecia* group and the *Pithecia monachus* group, and divided them into five species and six subspecies. However, the most recent taxonomic revision of the genus by Marsh (2014) divides the sakis into 16 species by reinstating a number of previously-recognized species, elevating some forms from the subspecies to species level, and describing five new species.

Sakis are medium size primates with adults weighting from 1.5 to 4 kg and having a total body length (head to tail) from 250 to 980 mm (Hershkovitz 1987, Marsh 2014, Norconk 2011). Sakis occupy a range of habitats varying from tropical rainforests to regions of relatively low rainfall, and occupy riverine, flooded, and *terra firme* forests (Norconk 2011, Norconk & Setz 2013). Like titi monkeys, saki monkeys have also been reported as having a monogamous mating system, mainly because they are often reported as living in small social groups including a single male-female pair-mate, though as noted above, this conflates the ideas of grouping pattern and mating behavior (Aquino et al. 2009, Di Fiore et al. 2007, Fernandez-Duque et al. 2013). However, they appear to show greater variation in group size and structure than titi monkeys do, since some researchers have described groups of sakis that do not match the typical one adult male:one adult female ratio expected for pair-bonded primates (Lehman et al. 2001, Norconk 2011, Norconk & Setz 2013). Among sakis, males and females do not engage in as much social contact or coordination of activities as titi pairmates, and male sakis provide little or no direct care for offspring (Fernandez-Duque et al. 2013, Kleiman 1977, Norconk 2011, Norconk & Setz 2013, Wright 1986).

SOCIAL AND GENETIC MONOGAMY

The occurrence of monogamous social systems among mammals has been considered a puzzle for evolutionary biologists and biological anthropologists for more than 40 years (Kappeler 2013, Kleiman 1977, Lukas & Clutton-Brock 2013). Early on, monogamy was defined as a pair-mate relationship where the male and female enjoy almost exclusive mating access to one other (Kleiman 1977). However, as the study of mating systems encompasses both behavioral patterns and their genetic outcomes, monogamy is currently studied both in terms of male-female living arrangements (i.e., "social" monogamy) and in terms of actual breeding exclusivity (i.e., "genetic" monogamy) (Griffith et al. 2008, MacManes 2013, Reichard 2003). Social monogamy can be defined as a high affiliation and social tolerance of pair-mates where the breeding pair shares a common range or territory, presents a relatively high degree of synchronization in their behavior, and associates with each other for more than one breeding season (Anzenberger 1988, Fernandez-Duque et al. 2000, 2013; Lukas & Clutton-Brock 2013, Mock & Fujioka 1990). Moreover, diverse authors have demonstrated that social monogamy is not a strict phenomenon, but rather it might exhibits a complex variation in the patterns of sociality (Fernandez-Duque et al. 2013, Porter et al. in review).

Various studies have discussed the possible evolutionary factors that might have influenced the evolution of monogamous mating systems in spite of the reproductive advantage mammalian males would seemingly enjoy by having access to multiple mates. Paternal care, infanticide avoidance, and the distribution of females in space are some of the factors speculated to have influenced the origin or maintenance of monogamous mating systems (Kleiman 1977, Lukas & Clutton-Brock 2013, 2014; Opie et al. 2014). However, several field studies have demonstrated either the absence of parental care in

monogamous taxa (Brotherton & Manser 1997, Fernandez-Duque et al. 2013) or the existence of direct parental care in non-monogamous mating systems across mammals (Kleiman & Malcolm 1981). Additionally, in a recent meta-analytic study of more than 2500 species of mammals of all orders, Lukas & Clutton-Brock (2013) concluded that the need for paternal care and the risk of infanticide are unlikely to have been the dominant selective forces favoring the evolution of monogamy among different mammalian taxa.

Whether social monogamy reflects genetic monogamy is thus an important question that arises when studying socially monogamous primates. Despite a prolonged and stable social relationship among pair-mates, reports of extra-pair copulations among socially monogamous primates are not rare (Barelli et al. 2013, Bonadonna et al. 2014, Palombit 1994, Reichard 2010). These findings suggest that animal social organization is a poor indicator of the genetic mating system raising questions about the role of extra-group-paternity in the evolution of mating systems, how common extra-group copulations are, and how they correlate with intraspecific variation in social organization.

MICROSATELLITE MARKERS AND THE STUDY OF GENETIC MATING SYSTEMS

While the evolution of mating systems and sexual selection theory have been topics of major interest in evolutionary biology for more than four decades (Alexander 1974, Kleiman 1977, Kleiman & Malcolm 1981, Lukas & Clutton-Brock 2013, Orians 1969, Selander 1965), parentage analyses based on genetic data have received less attention (Akçay & Roughgarden 2007, Chapman et al. 2013, Hauver et al. 2010, Huck et al. 2014). Even when a species can be characterized as "socially monogamous" based on behavioral data demonstrating strong pair bonds, territoriality, mate guarding, and intense male parental care, its genetic mating system may still be characterized by multi-male,

multi-female mating or genetic promiscuity, as has been commonly observed in birds (Akçay & Roughgarden 2007, Petrie et al. 1998). Thus, the development of genetic tools like marker-based parentage analysis for studying breeding systems is an open and important area of research.

Microsatellite markers, also referred as short tandem repeats (STRs), are short repeating motifs of 2 to 6 base pairs and are widely distributed in the genome (Ellegren 2004). Microsatellites are among the most variable regions of the genome because they have a high mutation rate (Brinkmann et al. 1998, Li et al. 2002). As a consequence, STR regions are typically highly polymorphic among individuals of the same population and are thus extremely useful for parentage analyses, genetic mapping, and genetic structure analyses (Brinkmann et al. 1998, Ellegren 2004, Guichoux et al. 2011, Sun et al. 2012). Using STR markers for parentage studies is also advantageous because microsatellites commonly present a high success rate for cross-amplification in closely related species, their accuracy is easy to determine because a large proportion of errors can be identified in pedigree analyses when there are many alleles for each locus (Guichoux et al. 2011, Li et al. 2002, Schlötterer 2004).

The use of polymorphic microsatellite markers for parentage assessment and the genetic study of mating systems is becoming more and more common in wild (Czarnomska et al. 2013, DiBattista et al. 2012, Garrigue et al. 2004, Hasegawa et al. 2015, Moen et al. 2004, NyströM et al. 2012) and captive or domesticated (Li et al. 2010, Regidor-Cerrillo et al. 2013, Selvamani et al. 2001, Souza et al. 2012) populations of a variety of organisms, including primates (Barelli et al. 2013, Huck et al. 2014, Kolleck et al. 2013, Morin et al. 1998, Strier et al. 2011, Sukmak et al. 2014, Van Belle et al. 2012). Additionally, numerous studies have described the development of new genetic markers or have tested the use of previously developed markers in additional primate species (e.g.,

Babb et al. 2011, Cortés-Ortiz et al. 2010, Di Fiore & Fleischer 2004, Merker et al. 2012, Oklander et al. 2006, Xu et al. 2013).

Here, I describe screening of a set of makers known to be polymorphic in other New World primates in a wild population of titi and saki monkeys from the western Amazon that has been the subject of long-term observation. The identification of a panel of polymorphic genetic markers for these species is necessary for future studies of parentage, reproductive success, and kinship, as well as for assessing the study populations' genetic structure and dispersal behavior. Overall, in this study I screened 13 microsatellite loci and evaluated their utility for parentage determination, and I discuss their use for future genetic analyses of population genetic structure.

Methods

STUDY POPULATIONS

In this study, I collected and analyzed data from a population of *Callicebus discolor* (Figure 1) and *Pithecia aequatorialis* (Figure 2) living in the Tiputini Biodiversity Station (TBS, 76° 08' W, 0° 38' S), which is located in the Yasuní National Park and Biosphere Reserve in Ecuador. The TBS preserves a tract of ≈650 ha of primary Amazonian rain forest along the Tiputini River in eastern Ecuador. Besides *C. discolor* and *P. aequatorialis*, TBS is home to eight other genera of primates (Di Fiore and Fleischer 2005, Romoleroux et al., 1997).

Equatorial sakis and red titi monkeys at TBS have been studied and observed regularly since 2003 as part of a long-term project on the comparative socioecology of monogamous platyrrhines (Carrillo-Bilbao et al. 2005, Di Fiore et al. 2007, Fernandez-Duque et al. 2008).

SAMPLE COLLECTION AND DNA EXTRACTION

Animals were darted and captured via remote injection with an anesthetic using a tranquilizer dart fired from a CO_2-powered rifle according to procedures used by Di Fiore et al. (2007) and Fernandez-Duque et al. (2008). Captured animals were measured and had a tissue sample collected from the ear. For this study, I analyzed samples from 17 red titi monkeys (Figure 1) and 10 sakis (Figure 2). After collection, tissue samples were stored in RNALater solution (Ambion) or in a homemade nucleic acid preservation (NAP) buffer (Camacho-Saenz et al., 2013). Samples were maintained at room

temperature until they could be transported out of the field site. Then they were exported to the Primate Molecular Ecology and Evolution Laboratory at the University of Texas at Austin and stored at -20°C until DNA extraction. I extracted high quality genomic DNA from the tissue samples using the DNeasy® Blood & Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol.

MICROSATELLITES AND PCR-BASED GENOTYPING

I analyzed a set of 13 microsatellite marker loci already isolated from either humans or other New World primates (Table 1). These markers were screened on genomic DNA from the 17 red titi monkeys and 10 sakis individuals to determine: (i) whether they would amplify reliably, (ii) whether they are polymorphic, (iii) if they are in Hardy–Weinberg equilibrium (HWE), and (iv) the estimated exclusionary power of the parentage determination. I also used the genotypes generated to conduct preliminary analyses of parentage in several putative family groups of each species.

The first PCR screening reactions were made in a final volume of 8µL including: 4.0 µl of 2X QIAgen multiplex PCR master mix, 1.6 µl of the primer mix, 0.9 µl of water, and 1.5 µl of unquantified DNA extract diluted in water at proportion of 1:5. I used fluorescently-labelled forward primers (5' labeled with 6-FAM, HEX, or NED dyes) and nonfluorescent reverse primers. Subsequent PCR replicates were carried out in a total volume of 5 µl which included: 2.5 µl of 2X QIAgen multiplex PCR master mix, 1 µl of the primer mix, 0.5µl of water, and 1 µl of unquantified DNA extract diluted in water at proportion of 1:5.

The cycling conditions for all PCR reactions were as follow: 15 minutes of initial denaturation at 95 °C; 37 cycles of 94 °C for 30 seconds (denaturation step), 55 °C for

1.5 minutes (annealing step), 72 °C for one minute (extension step), and 60 °C for 30 minutes (final extension). The initial PCR products were visualized via electrophoresis in 1% agarose gels in order to confirm successful amplifications. Then, fragment sizing was carried out on an ABI 3730 DNA Analyzer using GeneScan 500-ROX size standard (Applied Biosystems) at the DNA Sequence Facility of the Institute for Celular and Molecular Biology of the University of Texas at Austin.

Genotypes were read and scored using the software GeneMapper 4.0 (Applied Biosystems), with all allele calls subsequently confirmed by visual inspection. To minimize possible genotyping errors due to allelic dropout, we replicated heterozygous genotypes at least twice and homozygous genotypes at least four times (Morin et al. 2001, Soulsbury et al. 2009).

MICROSATELLITE PARAMETERS AND PARENTAGE ANALYSIS

I used Arlequin v3.11 (Excoffier et al. 2005) to calculate the expected (H_E) and observed (H_O) heterozygosity and to test for deviation from expected Hardy–Weinberg genotype proportions (HWP) and for linkage disequilibrium among all pairs of loci. As Arlequin uses a version of the Markov-chain exact test to detect significant deviations from HWP, it is more appropriate for small sample sizes (Guo & Thompson 1992). In Arlequin, I used the likelihood ratio test to detect linkage disequilibrium (Slatkin & Excoffier, 1996). However, considering the small sample size, I also estimated the physical locations of each microsatellite locus by using the megaBLAST algorithm (Morgulis et al. 2008) to search for the DNA sequence published in Genbank (accession number from original publications – Table 1) for each microsatellite locus and specifying

the complete genomes for *Callithrix jacchus*, *Macaca mulatta*, and *Homo sapiens* as the Search Set, as proposed by Babb et al. (2011).

For titi monkeys, I performed maternity and paternity analyses for 8 infants (5 females and 3 males) using 3 sampled adult females as possible dams and 6 sampled adult males as possible sires (Figure 1). For equatorial sakis, I conducted maternity and paternity analyses for 3 infants (2 males and 1 female) using 2 sampled adult females as candidate dams and 5 sampled adult males as candidate sires (Figure 2). Candidate dams and sires included those that were suspected to be the offspring's mother and father based on behavioral observations (i.e., "expected parents") as well as all other adult females and males present in the population for whom a tissue sample was available.

Parentage exclusion probabilities, polymorphism information content (PIC), average non-exclusion probability for identity, and maternity and paternity tests were calculated using the software Cervus v3.0.3 (Marshall et al. 1998, Slate et al. 2000) and applying a Bonferroni correction for multiple tests (Rice 1989). This software uses the allele frequencies of all individuals sampled to calculate the log-likelihood score (LOD) for each candidate parent, which is the likelihood of paternity or maternity of a particular adult male or female, respectively, relative to the likelihood of paternity or maternity of an arbitrary male or female in the population (Marshall et al. 1998). I first conducted both paternity and maternity tests separately, allowing Cervus to choose the most likely father and mother for each infant from among the full set of candidate sires and dams. I then conducted both paternity and maternity analyses a second time, incorporating information about the identity genotypes of the dams and sires inferred from field observations. Finally, I conducted a parent pair analysis by allowing Cervus to choose the most likely combination of sire and dam from any adult male and adult female candidates at the same time.

The most likely sire and dam for each offspring are those with highest LOD values among the set of candidates. The confidence level assigned to the most-likely dam or sire is based on the difference in LOD scores between the most-likely parent (LOD1) and the next most-likely parent (LOD2). Cervus calculates the required difference in LOD scores to a particular assignment of confidence (e.g., 80%, 95%) by comparing the difference in LOD scores between the true parent and the distribution of LOD values for other candidate parents found in a parentage simulation run with allele frequencies like those seen in the population and assuming a user-defined proportion of all possible candidates were sampled and a user-defined genotyping error rate. For my analysis, I simulated 10,000 offspring and I used the default mistyping error rate of 1%. Considering the small sample size, I performed all analyses twice, assuming that my sample included first 60% and then 80% of potential dams and sires.

Results

TITI MONKEYS

Among the 13 tested loci, 12 successfully amplified in titi monkeys and 11 were polymorphic. Although the Leon2 locus presented two alleles, it was not included in the subsequent analyses because one of the alleles detected was present in only one individual (an infant), who was heterozygous. As this allele appeared in four independent PCR reactions, it could be either a new mutation or consequence of the presence of allelic dropout in either the offspring's dam or sire. The final panel of 10 polymorphic markers identified for titis (Tables 2 and 3) had a mean observed heterozygosity (H_O) of 0.629 (ranging from 0.294 to 0.941), a mean number of alleles (k) of 5.5 (ranging from 2 to 9), and a mean polymorphic information content (PIC) value of 0.59.

The combined probability of identity (CNE-I, or the combined probability of two independent samples having the same identical genotype) and the combined probability of identity of siblings (CNE-SI), using all 10 polymorphic microsatellites, were 7.1E-9 and 5.2E-4, indicating a very low probability that any two individuals or any two full siblings, respectively, chosen at random would exhibit the same multilocus genotype given allele frequencies in the population. The observed heterozygosity at each locus did not depart significantly ($P>0.05$) from that expected under Hardy–Weinberg equilibrium for all loci. Additionally, the estimated frequency of null alleles ranged from -0.152 to 0.157 across the different markers.

A likelihood ratio test indicated significant linkage disequilibrium (LD) for several pairs of loci (Apm01-D17S804/ LL157/ LOCUS5; D8S260-LL1118/ SB30; Leon21-LL157; LL1118-LL157/SB30; LL157-SB38; and LOCUS5-SB30). However, the

comparative alignment of each locus to the genomes of other primates (Table 4) suggests that the loci for titi monkeys, and specifically those pairs of loci identified as possibly under LD, are most likely to be located in different chromosomes. Additionally, all pair of loci indicated as having a significant LD were located in different chromosomes in *C. jacchus* or *M. mulata*. This suggests that the test I used to detect LD could be flawed or inappropriate, perhaps due to the small sample size. The megaBLAST alignments matched the loci for *C. discolor* panel to a minimum of 6 different chromosomes in *C. jacchus*, 7 chromosomes in *Macaca mulatta*, and 6 chromosomes in *H. sapiens* (Table 4).

The 10 polymorphic loci, combined, provided a parentage exclusionary percentage of 96.7% when both parents were unknown, and 99.7% when one parent was known. The parentage analysis I conducted successfully assigned maternity for 4 and paternity for 8 out of 8 infants (Table 7). Values of LOD for maternity ranged from 1.97 to 8.27. However, 4 infants had no mother assigned, including 3 with suspected mothers not included in the analysis. All candidate dams, except those assigned as the most likely mother, were successfully excluded from being possible dams with at least 3 loci mismatches. LOD values for candidate sires ranged from 1.65 to 7.64. Paternity for six infants was assigned to the expected sires and for two infants was assigned to an unexpected adult male. Bandito, an infant born in 2009, was the only infant for whom the second most likely candidate sire was not excluded from parentage based on multiple mismatches. However, when the paternity analysis was run with the identity of the most likely dam specified, all other candidate sires, except the most likely one, were excluded from being a candidate sire with 2 loci mismatching among the triad of dam-sire-and-offspring. In the parentage analysis where I asked Cervus to identify the most likely mother and father simultaneously, parentage was assigned to 4 infants (LOD scores of 1.84, 6.95, 7.96, and 9.84), all of them in accordance with expectations from field

observations. The results for the parentage analysis for titi monkeys were the same regardless of whether I used 60% or 80% as the estimated proportion of candidate dams and sires included in the sample.

SAKI MONKEYS

For the saki monkeys, 12 microsatellite markers out of the 13 I tested amplified successfully and were polymorphic (Tables 5 and 6). The final panel of 12 polymorphic markers screened in all individuals yield a mean H_O of 0.731 (ranging from 0.444 to 1), a mean number of alleles of 5 (ranging from 2 to 8), and a mean PIC value of 0.636. The CNE-I and CNE-SI were 3.89E-11 and 5.71E-5, respectively, again demonstrating a very low probability that any two individuals or any two full siblings chosen at random would exhibit the same multilocus genotype. Observed heterozygosity at each locus did not depart significantly ($P>0.05$) from that expected under Hardy–Weinberg equilibrium, except at the Locus5 marker, which suggested a significant deficit of heterozygous individuals. Given the small sample size for saki monkeys, it was not possible to estimate the null allele frequency for each locus.

Significant linkage disequilibrium (LD) was identified for the pair of loci Leon2 and SB38 using a likelihood ratio test. However, a comparative alignment showing the physical mapping of these markers across primates (Table 4) suggests that these loci in sakis are also likely to be located in different chromosomes. The megaBLAST alignments matched the loci for *P. aequatorialis* panel to a minimum of 6 different chromosomes in *C. jacchus*, 9 chromosomes in *Macaca mulatta*, and 8 chromosomes in *H. sapiens* (Table 4).

The combined parentage exclusionary percentages yielded by the panel of markers for saki monkeys were 98.5% when both parents were unknown, and 99.94% when one parent was known. My parentage analysis for saki monkeys successfully assigned maternity and paternity for 3 and 1 out of 3 infants, respectively (Table 8). LOD values for the maternity analysis were 1.21, 5.86, and 6.33, with maternity in all cases assigned (to the same, expected female) at the 95% of confidence level. A second candidate dam from the population was successfully excluded from being the possible dam with at least 5 loci mismatching. Only one infant had an adult male indicated as the most likely father in the paternity test. This paternity assignment had a LOD score of 5.45 and was also assigned at 95% of confidence. All candidate sires, except that assigned as the most likely father, were successfully excluded from paternity by at least 5 mismatches between the male and the infant. In the parentage analysis for the most likely dam and sire simultaneously, the same parentage was inferred for the infant who had the most likely father determined in the paternity test alone (LOD score of 1.18 and 95% of confidence), in accordance with expectations from field observations. For saki monkeys, the results for parentage analysis were the same regardless of whether the analysis was run presuming that either 60% or 80% of putative parents were sampled.

Discussion

This study identified a panel of polymorphic microsatellite loci suitable for investigating relatedness and genetic mating systems in wild titi and saki monkeys. Heterozygosity and the polymorphic information content value presented here for both titi and saki monkeys are slightly higher than H_E and PIC presented in similar studies with *Aotus* (Babb et al. 2011), *Leontopithecus* (Grativol et al. 2001, Perez-Sweeney et al. 2005), and different species from the genus *Alouatta* (Cortés-Ortiz et al. 2010, Oklander et al. 2006, Van Belle et al. 2012), and are more comparable to those presented for *Lagothrix poeppigii* (Di Fiore & Fleischer 2004, 2005) and *Macaca mulatta* (Xu et al. 2013). However, these panels yielded a slightly lower number of alleles per locus on average, which is not surprising given the relatively small number of individuals sampled. The parentage exclusionary rates presented by both titi and saki panels were high and comparable with the exclusionary power offered by panels used in other studies of wild primates (Babb et al. 2011, Di Fiore & Fleischer 2004, Oklander et al. 2006, Xu et al. 2013).

Although the parameters found in both titi and saki monkeys panels were promising, they have to be interpreted carefully because of my small sample size (17 titi monkeys and 10 sakis). The sample size needed to correctly estimate the parameters of a loci panel (e.g. H_O, H_E, CNE-I, and CNE-SI) is one common issue when conducting population genetic studies. Some researchers argue that the minimum sample size needed is related primarily to the number of individuals, which affects one's ability to estimate all of the alleles present in each population (Kalinowski 2005). However, other researchers have argued that accurately estimating allele frequency and diversity are

more important than detecting all alleles (Hale et al. 2012). These researchers have demonstrated that a sample size of 20 to 30 individuals is typically needed to get reliable allele frequency estimates, especially for large populations (Hale et al. 2012, Pruett & Winker 2008). However, Pruett & Winker (2008) have also shown that for small sample sizes (5 to 10 individuals), the mean and standard error are likely to encompass the true values for average H_E and H_O. Thus, despite the small number of individuals I used, both panels of loci for titis and saki monkeys are informative and likely provide a reasonable estimation of the genetic diversity.

Different factors, such the number and allelic diversity of loci studied (Bernatchez & Duchesne 2000, Harrison et al. 2013, Nielsen et al. 2001), the proportion of the population sampled (Marshall et al. 1998, Oddou-Muratorio et al. 2003), genotyping errors, mutations, and the presence of null alleles (Hoffman & Amos 2005), all affect the success with which genetic studies can be used to evaluate parentage. Among these, a limited number of markers with low allelic diversity and incomplete samples of all candidate parents appear to have the largest effect on the accuracy of parentage assignments (Marshall et al. 1998). Using simulated models, Harrison et al. (2013) investigated how different factors might affect the accuracy of diverse parentage inference methods. They found that the most important factor influencing the success of parentage analysis is the number and diversity of loci. The proportion of candidate parents sampled also had a small but significant impact, which could be minimized in all simulations by increasing the number and diversity of loci used (Harrison et al. 2013). Based on a simulation study, Bernatchez and Duchesne (2000) have argued that, in many cases, only a relatively low number of loci need to be screened to achieve high success in parentage assignment even when the population contains a relatively large number of possible parents, although their models utilized a panel of loci with 13 alleles on average,

which is much more than the allelic diversity of my panels for titi and saki monkeys. Similarly, Nielsen et al. (2001) demonstrated that 10 to 14 loci (with an average of ~4 allele per locus) are necessary to reliable infer paternity using a Bayesian approach on simulated data.

Although some studies of population genetics, mating systems, kinship, and relatedness used microsatellite panels with a higher number of loci (16 loci for spider monkeys - Di Fiore et al. 2009, 14 loci for azara's owl monkeys - Huck et al. 2014, 21 loci for chimps - Mitchell et al. 2015, and 19 loci for black howler monkeys - Van Belle et al. 2012), other studies have successfully conducted parentage analysis on populations of wild nonhuman primate also used panels with as few as 8 (woolly monkeys - Di Fiore et al. 2009), 10 (rhesus macaques - Xu et al. 2013) or 11 (black and gold howler monkeys - Oklander et al. 2006) markers. The loci I found reliably amplifying and being polymorphic for titi and saki monkeys generated panels with 10 and 12 microsatellite markers, respectively. These panels constitute a reliable source of STR markers for future parentage, mating system, or other population genetic studies of *Callicebus* and *Pithecia* species.

In my preliminary parentage analysis, I performed separate paternity, maternity, and simultaneous parentage assignments for 8 titi monkey and 3 saki offspring. For titi monkeys, field observations identified both an expected dam and sire for 5 of these infants, and my genetic analyses corroborated field expectations for 4 of these offspring, all with 95% confidence. For one of titi offspring (Baleia), however, no female was assigned as a most-likely dam and the male with highest LOD score (95% confidence) was incongruent with preliminary field expectations. Nevertheless, when field observations started, Baleia was a subadult female and it is plausible that she may have been a dispersing transient individual, rather than daughter of the adult pair in the group

in which she was sampled (*Callicebus* group B). Moreover, Baleia's most likely sire (Luciferus from *Callicebus* group L) was the resident male in the group next to where she was sampled, suggesting she could be dispersing from one adjacent group. For the remaining three *Callicebus* offspring, samples were available for only the suspected father and not the suspected mother. Not surprisingly, for each of these offspring, no most-likely dam was assigned, but for two, the male suspected from field observations to be the father was identified as the most-likely sire, while for one the most likely sire identified from the parentage analysis was incongruent with field observations.

Among the *Pithecia* offspring, observational data were only sufficient to suggest one expected mother-father-offpring trio among the animals sampled, and the genetic data indeed assigned a most likely dam and sire in accordance with the field expectations. For the other two infants, only the expected dam was sampled and, again, the genetic data corroborated that she was the most likely dam for both offspring (albeit from a very limited set of only 2 candidate dams).

Overall, this study identified 10 and 12 polymorphic microsatellite markers for titi monkeys and sakis respectively. All loci in both panels amplified reliably and were sufficiently polymorphic to suggest that these panels will be of general utility for characterizing genetic structure in wild populations of both species. Furthermore, genetic parentage results for a small number of *Callicebus discolor* and *Pithecia aequatorialis* offspring – while preliminary – indicate that both panels of loci will also be useful for future studies on mating systems, kin selection, relatedness, and other genetic studies of wild titi and saki monkeys.

Tables

Table 1. Detailed information of 13 microsatellite loci screened for *Callicebus discolor* and *Pithecia aequatorialis* in this study.

Locus Name	Isolated from	5′–3′ primer sequences used	Repeat motif in original seq.	Accession no.	Ref.
Apm01	*Alouatta paliata*	F: CACGTGTGTCCAGCTTGTCT R: ATTCTGCTGCCCTTGAGTTC	$[TG]_{25}$	GQ917118	1
D17S804	*Homo sapiens*	F: GCCTGTGCTGCTGATAACC R: CACTGTGATGAGATGTCATTCC	$[AC]_{18}$	Z17033	2
D5S111	*Homo sapiens*	F: GGCATCATTTTAGAAGGAAAT R: ACATTTGTTCAGGACCAAAG	$[CA]_{13}C[CA]_6T[AC]_5$	X54592	3
D8S165	*Homo sapiens*	F: ACAAGAGCACATTTAGTCAG R: AGCTTCATTTTTCCCTCTAG	$[AC]_{16}$	M94656	4
D8S260	*Homo sapiens*	F: AGGCTTGCCAGATAAGGTTG R: GCTGAAGGCTGTTCTATGGA	$[CA]_{25}$	Z16597	2
Leon2	*Leontopithecus chrysopygus*	F: CTGCTTCTTGTTCCACTTCTTCTC R: GTTTGGGTGGTTGCCAAG	$[CA]_{18}[CG][CA]_3$	AY706915	5
Leon21c75	*Leontopithecus chrysopygus*	F: CAGTTGAGGGAACAGGAATTA R: CACTGCACTGACAGAGCAAG	$[GT]_{19}[NA][GT]_5$	AY706920	5
LL 1-1#18	*Lagotrix lagotricha*	F: TTTCTCCCTCTCAGATTACCAG R: CCTTGAGGTTTTTGGGTTCC	$[CA]_2[TA]\ [CA]_{17}$	AY405290	6
LL 1-5#7	*Lagotrix lagotricha*	F: TGGCAAGTCTGGTTTCAAGC R: TTCCAGACTGAGCTAGGATGC	$[GA]_4[GT]_4[CT]$	AY405291	6
LL 1-1#10	*Lagotrix lagotricha*	F: GGTGAATGAGAGAATCAAAG R: TATGTTCCACAGTAGAAAGC	$[GT]_{20}$	AY450288	6
LOCUS5 (Lr.P2BH6)	*Leontopithecus rosalia*	F: TCTGTTTGAATCCCCAGTCC R: GCAGTCCCTCAAGGTTTTCT	$[CA_]_{19}$	AF320577	7
SB30	*Saguinus bicolor*	F: TAAAGTTAAGATTGGATTTCAC R: GCAGAAAAACCTAACAATACA	$[CA]_9[AT][CA]_{11}$	AF367993	8
SB38	*Saguinus bicolor*	F: GCCTCAATGGGTTTTAACC R: AGAACGAGTCTGTATCTTGA	$[CA]_{19}$	AF367994	8

1 - (Cortés-Ortiz, Mondragón, and Cabotage 2010); 2 - (Weissenbach et al. 1992); 3 - (Weber, Kwitek, and May 1990); 4 - (Weber and May 1989); 5 - (Perez-Sweeney et al. 2005); 6 - (Di Fiore and Fleischer 2004); 7 - (Grativol, Ballou, and Fleischer 2001); 8 - (Böhle and Zischler 2002)

F and R indicate forward and reverse primers, respectively.

Table 2. Characterization of 10 microsatellite loci polymorphic in titi monkeys (n=34 chromosomes assayed).

Locus	**Size range (base-pairs)**	**Na[1]**	**Ho[2]**	**H_E[3]**	**HWE[4]**	**PIC[5]**
Apm01	188-216	9	0.764	0.832	ns	0.788
D8S165	150-160	6	0.764	0.677	ns	0.621
D8S260	189-192	2	0.294	0.258	ns	0.219
D17S804	152-154	2	0.352	0.499	ns	0.367
Leon21	389-398	5	0.823	0.762	ns	0.7
LL 1-5#7	216-231	6	0.588	0.766	ns	0.704
LL 1-1#18	152-184	7	0.941	0.805	ns	0.757
LOCUS5	100-132	9	0.588	0.764	ns	0.71
SB30	89-109	7	0.647	0.77	ns	0.722
SB38	106-110	2	0.529	0.401	ns	0.314
	Average	5.5±2.71SD	0.629±0.2SD	0.653±0.19SD		0.59±0.38SD

1 – Number of alleles; 2 – Observed Heterozygosity; 3 – Expected Heterozygosity; 4 – *P-value* for the test for deviation of Hardy-Weinberg equilibrium (HWE); 5 - Polymorphic Information Content; ns: Indicates a no significant departure from HWE.

Table 3. Genotypes for the 10 polymorphic microsatellite loci for each titi monkey individual.

Ind.	Apm01	D8S156	D8S260	D17S804	LEON21
Baleia	198-216	150-158	189-189	152-154	389-393
Banana	207-207	150-158	189-192	152-152	391-398
Bandito	207-207	158-158	189-189	152-152	391-398
Bongo	205-207	152-158	189-189	152-154	391-391
Brumble	205-207	152-158	189-192	152-152	391-398
Buttercup	205-207	150-158	189-189	152-152	391-398
Homero	200-207	154-158	189-189	154-154	391-395
Huito	198-207	154-158	189-192	152-152	391-395
Kebec	203-207	150-158	189-192	152-154	395-395
Kelly	203-203	150-156	189-189	152-154	391-395
Kia	188-203	150-160	189-189	152-152	395-395
Kong	203-207	158-160	189-192	152-152	391-395
Liam	196-196	158-158	189-189	154-154	389-393
Luciferus	196-198	158-158	189-189	154-154	389-391
Lulu	196-200	158-158	189-189	154-154	391-393
Puma	200-207	150158	189-189	152-152	391-398
Saul	205-210	152-160	189-189	152-154	393-393

Ind.	LL157	LL1118	LOCUS5	SB30	SB38
Baleia	216-227	156-182	100-100	92-96	106-110
Banana	225-229	162-182	104-112	96-96	110-110
Bandito	227-227	160-182	104-104	96-96	106-110
Bongo	227-227	160-182	104-104	96-105	106-110
Brumble	227-229	162-182	104-104	96-96	110-110
Buttercup	227-229	162-182	104-104	96-96	110-110
Homero	223-231	152-184	104-112	99-105	106-110
Huito	225-225	160-182	100-100	89-102	106-110
Kebec	225-229	154-182	100-102	96-102	110-110
Kelly	229-229	152-184	104-132	99-109	106-110
Kia	225-227	154-182	102-104	96-96	110-110
Kong	225-225	152-182	100-104	89-96	106-110
Liam	216-216	156-156	100-116	92-105	106-110
Luciferus	216-227	156-182	100-100	92-102	110-110
Lulu	216-227	156-160	112-116	96-105	106-110
Puma	225-227	182-184	104-108	102-105	110-110
Saul	227-227	156-182	106-126	109-109	110-110

Table 4. Inferred chromosomal localization of microsatellite loci using in the *C. discolor* and *P. aequatorialis* panels based on megaBLAST search results.

Locus	***Callithrix jacchus***	***Macaca mulatta***	***Homo sapiens***
Apm01	-	chromosome 2	chromosome 3
D17S804	chromosome 5	chromosome 16	chromosome 17
D5S111*	chromosome 2	chromosome 6	chromosome 5
D8S165	chromosome 16	chromosome 8	chromosome 8
D8S260	chromosome 16	chromosome 8	chromosome 8
Leon2*	chromosome 7	chromosome 1	chromosome 1
Leon21c75	chromosome 5	chromosome 16	chromosome 17
LL 1-1#18	chromosome 12	chromosome 9	chromosome 10
LL 1-5#7	chromosome 2	chromosome 3	chromosome 7
LOCUS5	chromosome 13	-	-
SB30	chromosome 7	chromosome 12	chromosome 10
SB38	chromosome 12	chromosome 20	Chromosome 16

Data source: Genbank and megaBLAST alignment database indexing (Morgulis et al. 2008). The DNA sequences for Apm01 (for *C. jacchus*), and LOCUS5 (for *M. mulatta* and *H. sapiens*) failed to align to a single location for any of the genome assemblies examined. *Locus used exclusively for the sakis' panel.

Table 5. Characterization of 12 microsatellite loci polymorphic in saki monkeys (n = 20 chromosomes assayed).

Locus	**Size range (base-pairs)**	**Na[1]**	**H_O[2]**	**H_E[3]**	**HWE[4]**	**PIC[5]**
Apm01	196-207	4	0.777	0.647	ns	0.558
D5S111	166-172	4	1	0.764	ns	0.674
D8S165	146-152	4	0.666	0.738	ns	0.642
D8S260	204-216	4	0.777	0.647	ns	0.558
D17S804	161-175	6	1	0.81	ns	0.73
Leon2	192-200	5	0.555	0.712	ns	0.631
Leon21	375-393	5	0.555	0.732	ns	0.642
LL 1-5#7	186-188	2	0.555	0.424	ns	0.321
LL 1-1#18	142-154	5	0.777	0.673	ns	0.6
LOCUS5	117-139	8	0.444	0.836	0.002**	0.77
SB30	111-130	6	0.888	0.81	ns	0.73
SB38	131-146	7	0.777	0.856	ns	0.786
	Average	5±1.5SD	0.731±0.18SD	0.721±0.11SD		0.636±0.17SD

1 – Number of alleles; 2 – Observed Heterozygosity; 3 – Expected Heterozygosity; 4 – *P-value* for the test for deviation of Hardy-Weinberg equilibrium (HWE); 5 - Polymorphic Information Content; ns: Indicates a no significant departure from HWE; ** Indicates a significant departure from HWE.

Table 6. Genotypes for the 12 polymorphic microsatellite loci for each saki monkey individual.

Ind.	Apm01	D5S111	D8S165	D8S260	D17S804	LEON2
Dharma	205-207	166-172	146-146	208-210	161-167	196-200
Marigold	196-205	168-172	146-150	208-208	165-169	192-192
Mayer	205-207	166-170	150-150	208-210	171-175	194-198
Milo	196-205	168-170	146-152	208-210	165-171	192-192
Mona	196-205	170-172	150-152	204-208	165-169	192-192
Mondika	196-207	166-172	150-150	204-210	165-171	192-194
Morpho	202-205	168-172	146-148	204-208	165-171	196-196
Pipian	205-205	168-172	146-148	208-208	167-169	192-196
Sancho	205-205	166-172	146-152	208-216	165-171	192-198

Ind.	LEON21	LL157	LL1118	LOCUS5	SB30	SB38
Dharma	379-393	186-186	144-144	127-127	111-125	133-146
Marigold	387-387	186-186	144-144	129-131	111-113	139-142
Mayer	384-384	186-186	142-151	131-131	113-130	133-135
Milo	379-387	186-188	144-154	129-131	117-117	139-142
Mona	384-387	186-188	142-144	131-133	111-117	142-142
Mondika	384-384	186-188	144-151	131-131	117-130	133-142
Morpho	387-387	186-188	144-149	137-139	117-127	137-137
Pipian	375-387	186-188	144-151	117-117	111-117	131-146
Sancho	379-387	186-186	144-154	121-121	111-130	135-142

Table 7. Paternity and maternity assignments for titi monkey infants.

Offsp.[1]	SG[2]	Exp. Dam[3]	Exp. Sire[3]	MLD[4]	N_{MLD}/ N_{SLD}[5]	PD[6]	MLS[4]	N_{MLS}/ N_{SLS}[7]	PS[6]	Trio[8]
Bandito[a]	B	Banana	Bongo	Banana	0 / 3	*	Bongo	0 / 0	*	*
Baleia[b]	B	Banana	Bongo	No MLD	-		Luciferus	0 / 3	*	
Buttercup	B	Banana	Bongo	Banana	0 / 3	*	Bongo	0 / 1	+	*
Brumble	B	Banana	Bongo	Banana	0 / 4	*	Bongo	0 / 1	*	*
Huito[b]	H	NI	Homero	No MLD	-		Kong	0 / 1	*	
Liam	L	Lulu	Luciferus	Lulu	0 / 7	*	Luciferus	0 / 5	*	*
Kia	K	NI	Kong	No MLD	-		Kong	0 / 3	+	
Kebec	K	NI	Kong	No MLD	-		Kong	0 / 2	+	

[1]Offspring.
[2]Social Group.
[3]Expected dam (Exp. Dam) and sire (Exp. Sire), respectively, from field observations.
[4]Most likely dam (MLD) and most likely sire (MLS) from parentage analysis (higher LOD scores among parent candidates).
[5]N_{MLD}: Number of mismatches between the offspring and the Most Likely Dam; N_{SLD}: Number of mismatches between the offspring and the Second Most Likely Dam.
[6]Pair confidence for maternity (PD) and paternity (PS) from separated analyses.
[7]N_{MLS}: Number of mismatches between the offspring and the Most Likely Sire; N_{SLD}: Number of mismatches between the offspring and the Second Most Likely Sire .
[8]Trio confidence for the combined parentage analysis.
[a]Second most likely sire not excluded from being the possible sire.
[b]Cases when expected and estimated maternity or paternity are incongruent.
NI: Sample not included in the analysis
No MLD: Any likely dam assigned.
* 95% confidence
+ 80% confidence

Table 8. Paternity and maternity assignments for saki monkey infants.

Offsp.[1]	SG[2]	Exp. Dam[3]	Exp. Sire[3]	MLD[4]	N_{MLD}/ N_{SLD}[5]	PD[6]	MLS[4]	N_{MLS}/ N_{SLS}[7]	PS[6]	Trio[8]
Mondika	M	Mona	Mayer	Mona	0 / 6	*	Mayer	0 / 5	*	*
Milo	M	Mona	NI	Mona	0 / 6	*	No MLS			
Marigold	M	Mona	NI	Mona	0 / 5	*	No MLS			

See legend for Table 5.
No MLS: Any likely father assigned

Figures

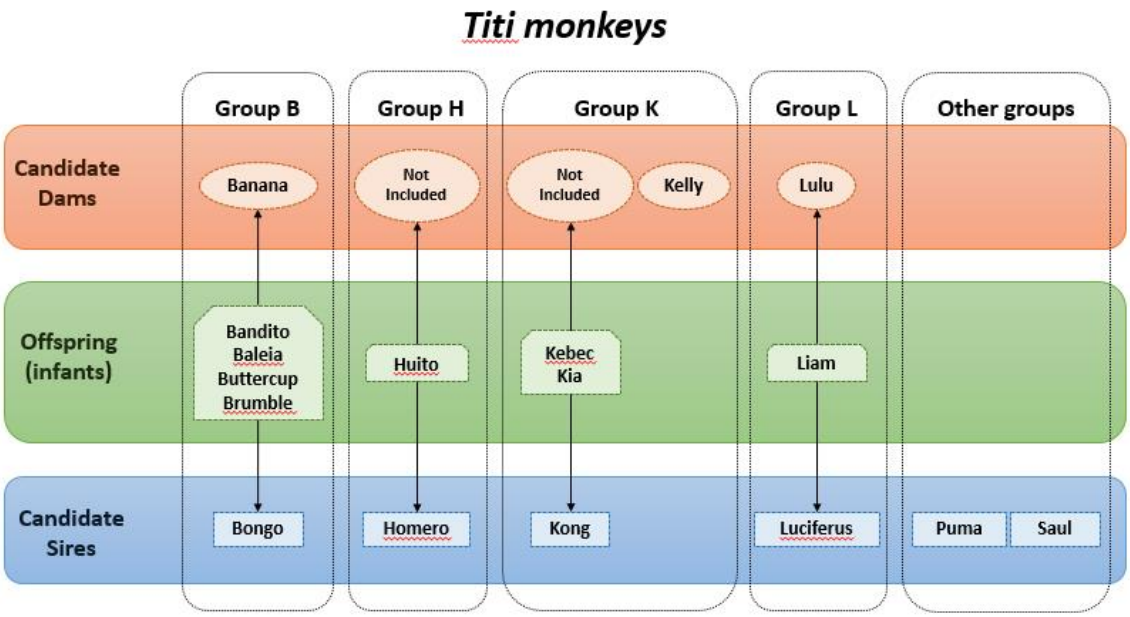

Figure 1. Group structure and relationship between all individuals of titi monkeys based on field observations.

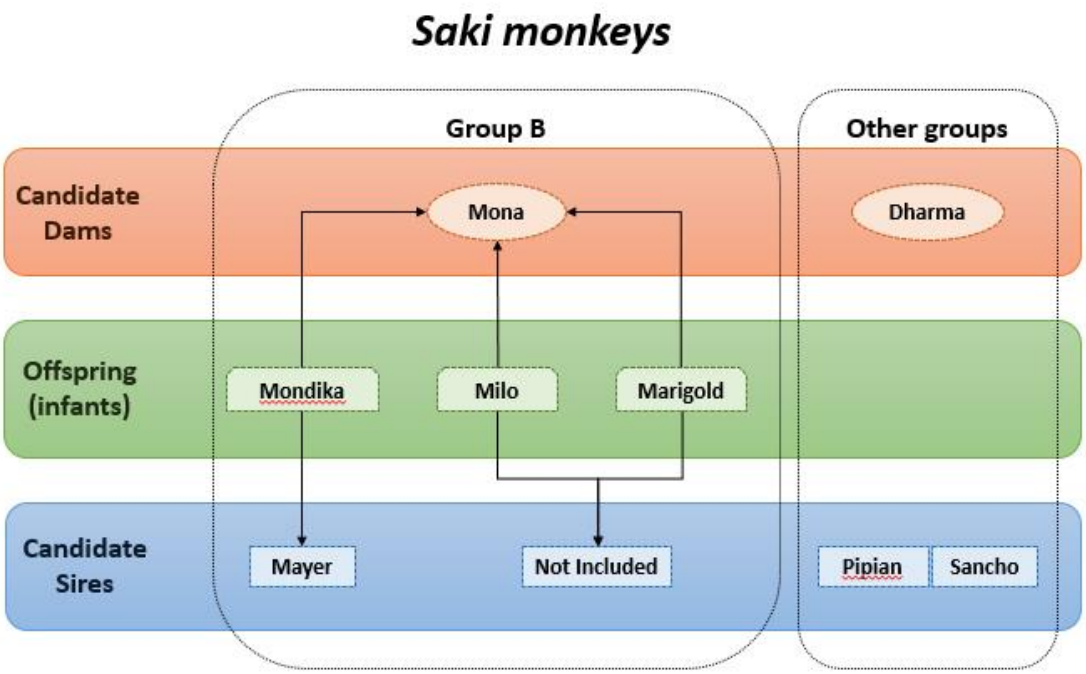

Figure 2. Group structure and relationship between all individuals of saki monkeys based on field observations.

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